SUPPLEMENTARY FIGURES

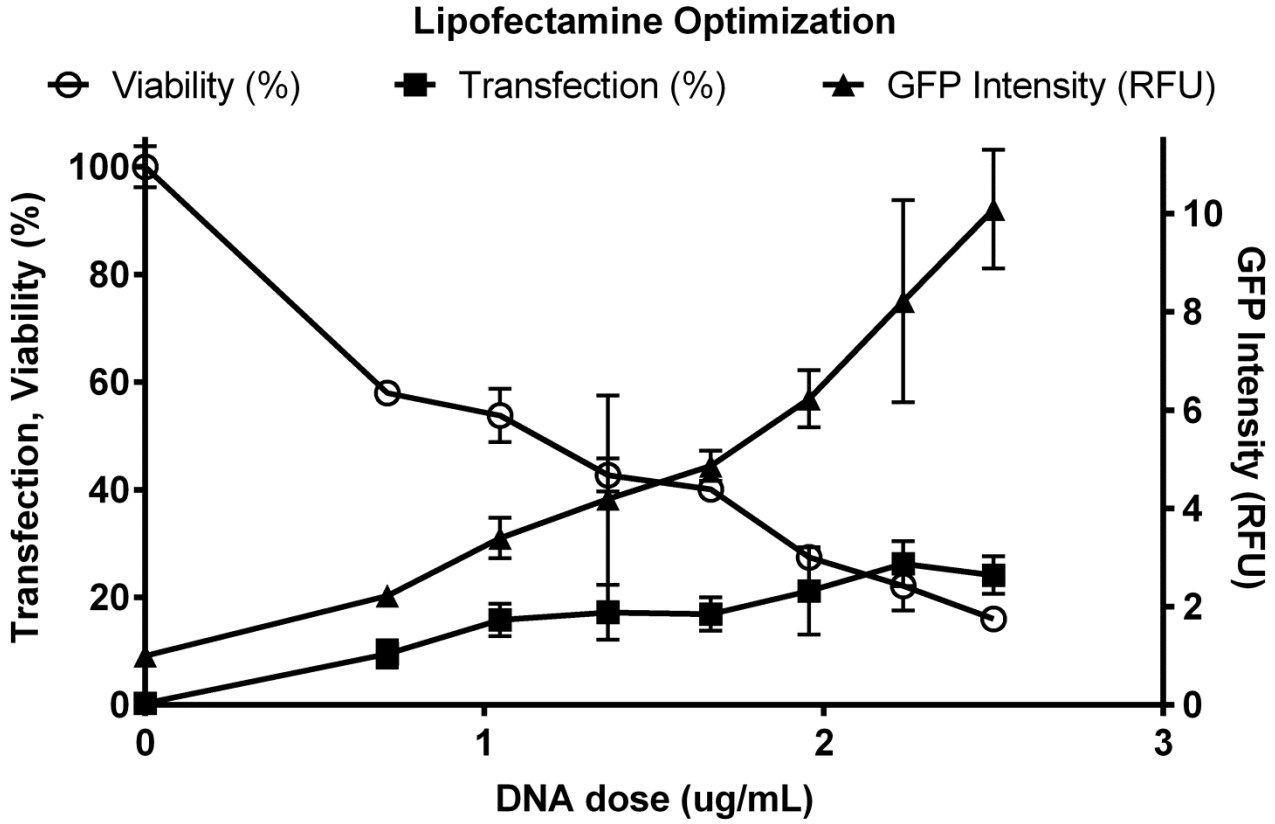

Figure S1. Lipofectamine optimization hAMSCs were transfected with Lipofectamine (TM) 2000 at a range of dosages. Cell viability was measured by MTS assay. Transfection efficacy was measured by flow cytometry. Each data point represents the mean +/- SEM of three points.

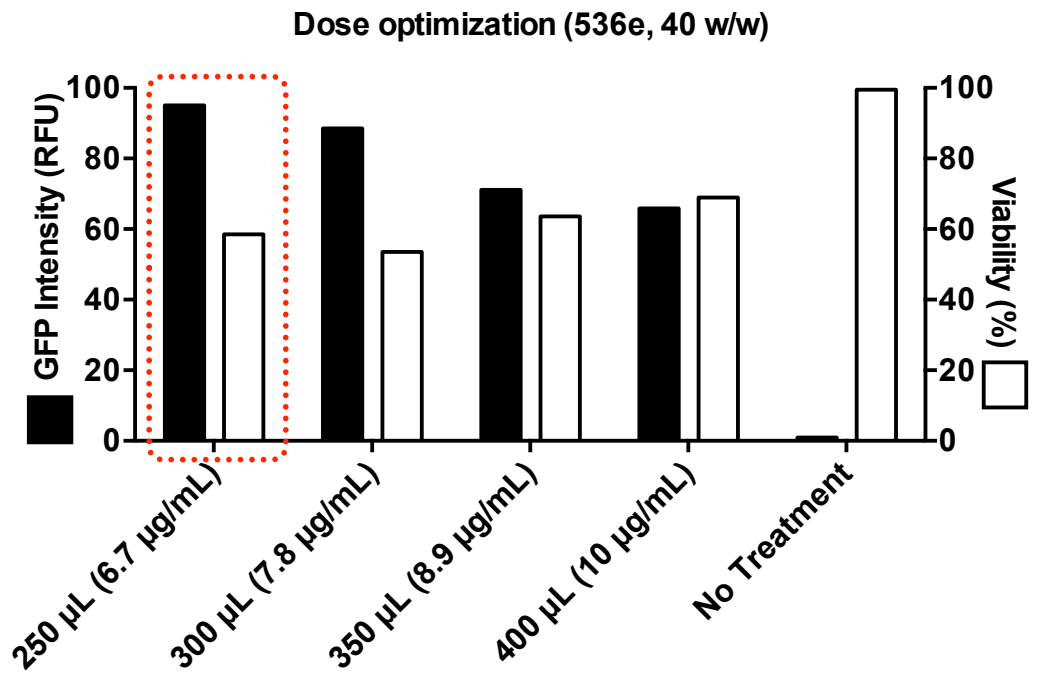

Figure S2. Dose optimization for 536e, 40 w/w. The figure shows the optimal doses of NPs for transfection of AdMSCs with 536e, 1.1:1, 40 w/w. The top formulation of 536e was tested for GFP expression and cell viability after 24 hr to optimize the DNA dosage. The top dosage of 250 µL NPs in 2 mL media (6.7 µg/mL DNA) was used for all further studies.

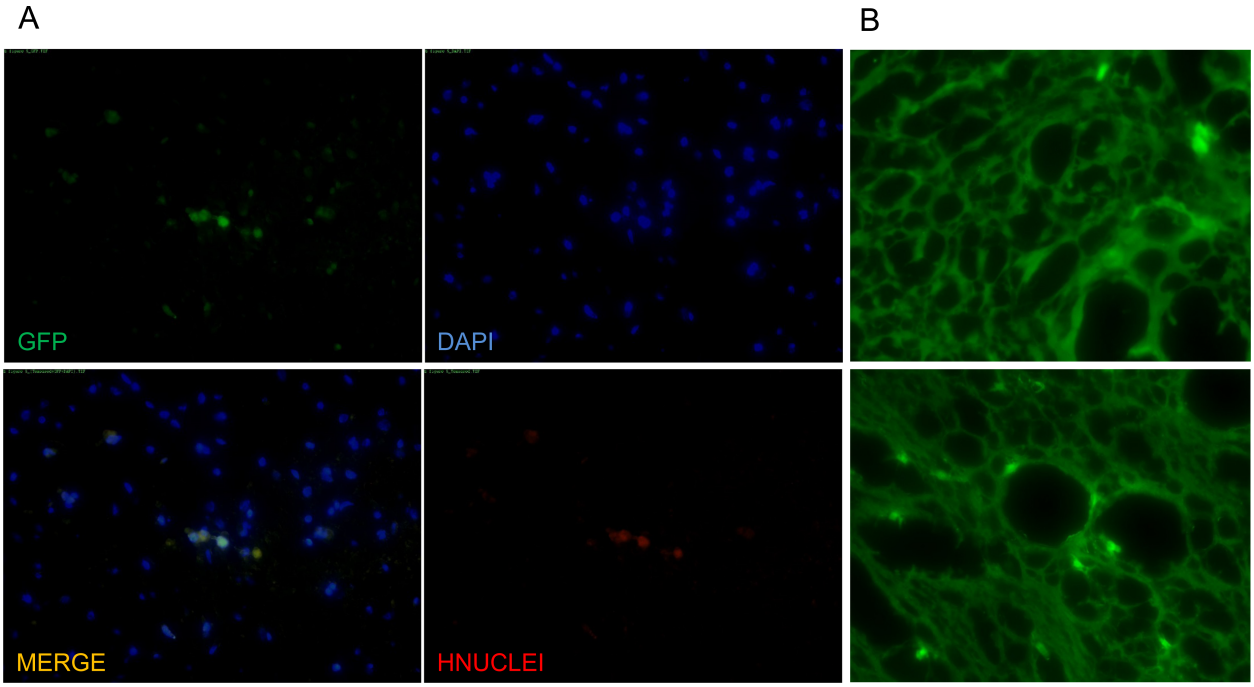

Figure S3. GFP/NP-hAMSCs can be visualized in the brains of U87-bearing rats after intranasal injection. (A) hNuclei^{+}/DAPI^{+}/GFP^{+} GFP/NP-hAMSCs are visible in the brain. (B) Confocal microscopy shows GFP^{+} hAMSCs in brain tumor tissue (40X magnification).

Movie S1. Migration Assay of hAMSCs, BMP4/LentiV-hAMSCs and BMP4/NP-hAMSCs

The movie shows representative cell motility video for each of three conditions: control (non-transfected), virus-transduced, and nanoparticle-transfected hAMSCs. Initially it shows three zoomed-in fields of view for each of the three conditions. Then, it shows each of these videos with 10 representative cells tracked from each. The cells were manually tracked and assigned a color that represents their instantaneous speeds, which were calculated based on the distance traveled from the previous frame. For every frame, each cell's current position (indicated with an asterisk) and path traveled (indicated with a solid line) were displayed. The instantaneous speeds were normalized such that the maximum speed of all three videos corresponds to dark red and a speed of zero (all cells at frame 1) corresponds to dark blue. The video then displays a representation of averaged speeds for each of the videos. For each cell, the average of all instantaneous speeds for the duration of the video was calculated. The color scheme was then normalized to the maximum and minimum average speeds across all three videos and the final location as well as path traveled for each cell was displayed in the corresponding color.

Table S1. List of the primers

Gene	Forward (5' - 3') primer	Reverse (5' - 3') primer
GAPDH	CACCCACTCCTCCACCTTTGA	TCCACCACCCTGTTGCTGTAG
eGFP	CACCATCTTCTTCAAGGACGAC	ACGTTGTGGCTGTTGTAGTTGT
BMP4	AAAGTCGCCGAGATTCAGGG	GACGGCACTCTTGCTAGGC

SUPPLEMENTARY MATERIALS AND METHODS

PCR

BMP-4 expression was quantified by qPCR analysis in the mesenchymal stem cells post-transfection with the optimal polymer identified. The mRNA was isolated from the cells using (Life Technologies) extraction method. RNA concentration was quantified by NanoDrop spectrophotometer (Thermo Scientific). cDNA was generated using iScript cDNA Synthesis Kit (BioRad) and amplified via SYBR Green PCR Master Mix and StepOnePlus Real-Time PCR System (Applied Biosystems). The C_T method was used to calculate BMP4 expression levels (See Table S1 for primers).

Western Blot

To investigate how much BMP4 was secreted from NP-hAMSCs, Western blot was performed using conditioned media from NP-hAMSCs seeded at 10^4 cells/cm^2 and cultured for 2 days, using known amounts of recombinant BMP4 as a standard curve for quantification. Twenty-five milliliters of culture medium (CM) was conditioned by 10^6 cells for 48 hr. Six milliliters of the total volume of CM was collected and concentrated via Amicon Ultra-15 centrifugal filter units with 10 kDa MWCO (Millipore) to a final volume of 450 μL. The same procedure was performed to collect CM with soluble factors and proteins released by non-transfected and GFP/NP-hAMSCs. Protein quantification was performed with the Pierce BCA Protein Assay Kit (Thermo Scientific). 25 μg of protein, corresponding to 26.05 μL of the concentrated sample or 1.4% of the initial CM volume, was subjected to electrophoresis on NuPage 10% Bis-Tris gels (Invitrogen) and transferred to Immobilon P^{SQ} PVDF membranes (Millipore) for 1.5 hr. The membranes were blocked for 1 hr in 5% milk (blotting grade blocker, BioRAD) suspended in

tris-buffered saline (TBS) with 0.1% Tween 20 (TBST). They were then incubated with primary antibodies against BMP4 (Mouse anti-BMP-4 Monoclonal Antibody, Millipore MAB1049) diluted 1:250 in 5% milk Primary antibody incubation was carried out either overnight at 4°C. The membranes were then washed in TBST before incubation with secondary antibody: Goat anti-Mouse IgG Antibody, (H+L) HRP conjugate (1:10000) for 1 hr at room temperature. After incubation, the membranes were washed again in TBST. Chemiluminescence was detected using the ECL Western Blotting Reagent detection kit (GE Healthcare).

Nuclear Magnetic Resonance

Proton Nuclear Magnetic Resonance (H^1-NMR) was performed to chemically characterize polymer product 536. H^1-NMR was completed using a 400 Hz Bruker UltraShield™, using deuterated chloroform ($CDCl_3$) as the solvent. The proposed chemical structure of polymer 536 shown in **Figure S1** was confimed (**Fig. S2**).

Gel Permeation Chromatography

Gel Permeation Chromatography (GPC) was performed to characterize the size distribution of synthesized polymer 536. GPC was performed using a Waters GPC using three Water Styragel columns in series (HR 1, HR 3, and HR4) and a Waters 2414 refractive index detector, both maintained at 40°C throughout the duration of the measurement. Polymer samples were loaded using a Waters 717 Plus Autosampler (Waters Corp., Milford, MA), using a polymer concentration of 5 mg/mL using GPC solvent (94% tetrahydrofuran, 5% dimethyl sulfoxide, and 1 piperidine by volume) as the eluent at a flow rate of 1.0 mL/min for 40 min. Polymer molecular weight distributions were calculated relative to polystyrene standards (Shodex, Japan).

Dynamic Light Scattering

The nanoparticle diameter and zeta-potential were measured via Dynamic Light Scattering (DLS) using a Malvern Zetasizer NanoZS. Nanoparticles were formed at the same concentrations and in the same manner as for transfections, and were then diluted 1:6 by volume in PBS and loaded into a disposable cuvette cell. Size, polydispersity index (PDI), zeta potential are reported in Figure 2.

Transmission Electron Microscopy

Transmission Electron Microscopy (TEM) was performed using a Philips/FEI BioTwin CM120 transmission electron microscope in order to image nanoparticles. 536 nanoparticles were formed as previously described for *in vitro* transfection assays, and 5 μL of the nanoparticle solution was allowed to dry onto a carbon-coated copper grid prior to TEM imaging. TEM images are displayed in Figure S4.